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Comment on bg-2021-8

Anonymous Referee #2

Referee comment on "Isolation of sub-pollen particles (SPPs) of birch: SPPs are potential carriers of ice nucleating macromolecules" by Julia Burkart et al., Biogeosciences Discuss., https://doi.org/10.5194/bg-2021-8-RC2, 2021

General Evaluation:

This is an interesting manuscript describing experiments regarding the preparation and release of molecular solutes and small subpollen particles (SPP) from birch pollen. Some of the results, for example the characterization of the SPP are novel, well described and interesting, while some other experimental procedures and results are described only poorly. Moreover, I am questioning some of the interpretation regarding the connection between SPP and ice nucleating molecules (INM), and whether the INM are proteinaceous or not.

The data presented in the current manuscript might be quite useful and, hence, may support publication. I do have, however, major concerns regarding some of the procedures and the interpretation of the experiments and their application to the atmospheric situation, see below. Moreover, in some places the manuscript is technically deficient, e.g. in the detailed description of procedures or results, sometimes making it hard to understand what the authors actually refer to. Also, the citations and reference list need more care.

Overall, I think that major revisions are certainly required, as outlined below, before the paper may become suitable for publication in Biogeosciences.

Scientific points:

1.) As far as I understand, cytoplasm generally contains also all kinds of soluble material such as proteins, lipids, polysaccharides, DNA, RNA and inorganic ions. Using the washing procedure in the extraction method described in Figure 2, I would assume that with more and more extraction volume in step 4, these solutes become more and more dilute in samples Cx. This is apparently the case for the INM. The authors also show a very slight decrease in CNC (meaning INM concentration) between samples A, B and C01. I am convinced one would see a very similar pattern with all other soluble materials, too, i.e. with proteins, with polysaccharides, with DNA, with ions etc. The authors of the current manuscript chose to investigate the content and concentration behavior of proteins using two methods (fluorescence and Coomassie staining), which is fine. And indeed, they found

a similar concentration behavior for the proteins when comparing samples A, B, and C01. (They did not analyze whether proteins decrease in the same way as do INMs in samples Cx, though.) From the concentration correlation between INM and proteins in samples A, B, and C01, they infer that the INM may be proteins.

But what if the INMs belong to another substance class, for example DNA or polysaccharides? (The latter has been suggested in previous publications, also by the current authors.) I would assume to observe a similar correlation: The INMs are somewhat diluted between samples A, B and C01. In that case, the correlation between proteins and INMs would be fortuitous and would just indicate a small dilution of (any kind of) soluble material from sample A, to B, and C01. Or another thought experiment: if the authors had chosen to measure the concentration of DNA rather than that of proteins, wouldn't they have observed a similar concentration trend for DNA, even if the INMs had been the proteins? That would not directly imply that the INMs are DNA, wouldn't it? So from my standpoint, the correlation between INM concentration and protein concentration is not a proof that the INMs are proteins. This fact should be stated more clearly in the conclusions and abstract, to avoid a false interpretation by readers. The sentence in lines 280-281: 'We highlight the possibility that the ice nucleation activity of Betula pendula pollen is linked not only to polysaccharides (Pummer et al., 2015) but also to proteinaceous INM.' goes in the right direction, but may not be enough.

2.) Lines 206-209: 'However, even after 1-hour ultrasonic treatment we did not find any ruptured pollen grains nor SPP (Figure S1). We believe that the usually applied extraction method, where pollen grains are only left in water and are then filtrated, do not actually yield SPP unless very fresh pollen grains are used. In this sense our method is unique and offers the possibility to study isolated SPP and gain further insight about the location of the INM within the pollen grain.'

These sentences suggest that in all previous studies on dried commercial pollen, SPP were not present in the washing water. Is this notion correct? Please make a clear statement. If so, doesn't this imply that the INM are NOT connected to the SPP as in previous studies INM were indeed found by simply washing the dried 'old' pollen. Please discuss in more detail.

3.) Lines 253-254: 'The signal correlates with heterogeneous ice nucleation of sample A, B and C01'. I am not sure I entirely understand what is meant by 'correlates' in this context. Simply, samples A, B, and C01 show fluorescence and they also show ice nucleation? Or the ice nucleation activity, namely CNC or T_50, correlates with the strength of the fluorescence signal? Please explain in more detail.

4.) Lines 271-272: 'In this study we develop an extraction method that gives access to the cytoplasmic material of pollen grains, even after the grains have lost the ability to germinate and rupture.'

While I applaud the authors for the realization and description of this extraction method for dried pollen, I am missing an analysis / a connection of the results presented here to the processes occurring in the atmosphere. The authors make a big point that the release of cytoplasmic material in fresh ('living') pollen is different from that of dried ones. How can they then make any quantitative conclusions and statements on free pollen and their release of ice nucleating material?

Moreover, in the sample preparation part (line 116: 'Freshly harvested pollen samples were collected from birch trees at the Danube Island in Vienna.'), the authors mentioned that they also collected fresh pollen in Vienna, but I did not see any comparative analysis or measurements of fresh with dried pollen. Why is that so? The authors could have made experiments with fresh pollen using the same SPP extraction procedure, using only steps 2-4, and then analyzing the filtrates in a similar manner. Why did they not do so?

5.) Lines 281-285: 'INM and SPP are both contained in the cytoplasm. The abundance of INM suggests that INM and SPP might not naturally separate in the atmosphere. SPP could act as carriers of INM…'

I was wondering whether the authors can really exclude that the observed INMs come from the outer part of the pollen. I again emphasize the fact that dried pollen release INM (as shown in previous publications), but not SPP (according to the authors' statements) contradicts the statement that INM and SPP are both contained in the cytoplasm. If INM come from the cytoplasm AND are released even without rupture, do we need to consider two different types of INM then? Please elaborate.

6.) I still have not understood whether the amount of washing water given in Figure 4 (and Figure S2 in the supplement) refer to cumulative volume values or not. For example, for sample C01, 1 mL of washing water was used, and hence sample C01 has a total volume of 1 mL. What about sample C02? Was another 1 mL of washing water used (cumulatively the second mL) and the total volume is again 1 mL? Or were 2 mL of water used for sample C02, giving a total volume of 2 mL, and making it cumulatively 2-3 mL of waters used. Similarly, is sample C70 10 mL in total volume with the cumulative 60-70 mL of washing water used (there is a sample C60 given in the supplement)? Please explain more clearly.

Along the same lines, I am not sure how the dilution factor in equation 1 was applied to the different sample Cx solutions, and also to the samples A, B, and D. If you use different water volumes for extraction/preparing samples A, B, Cx, and D, shouldn't the CNC concentration be quite different? Or was that volume taken into account in the dilution factor? If yes, which solution is the reference? Solution C01?

Minor and technical points:

7.) Apparently, the citations and references have not been assembled very carefully and need to be revised. Here are some examples:

Line 34: 'Mikhail Sofiev, 2013' This is an incorrect citation (given name should be removed), probably due to the fact that the author list is incorrect, too, see below.

Lines 43-44: 'on a global scale (Corinna Hoose et al., 2010; C. Hoose et al., 2010).' Apparently, these are two different references. Please indicate them correctly and use correct citations, e.g. Hoose et al. 2010a; Hoose et al. 2010b.

Line 365: The reference to Gute & Abbatt is incomplete.

Lines 367-368: The reference to Gute et al is incomplete.

Lines 403-405: The author list is corrupted. The correct author list is: Mikhail Sofiev, Jordina Belmonte, Regula Gehrig, Rebeca Izquierdo, Matt Smith, Åslög Dahl, and Pilvi Siljamo

Some references are missing their doi.

8.) Lines 48-49: 'The solution is then decanted and filtrated yielding what is called pollen washing water. The washing water is shown to induce ice formation at similar temperatures as the entire pollen grains.' The tense should be past, not present.

9.) Lines 90-91: 'For example, birch pollen grains were shown to germinate on leaves after light rain and release starch granules.' This sentence needs a reference.

10.) Lines 155-56: Please provide more information on the emulsion preparation, i.e. the type of paraffin and the concentration (ratio) of the lanolin.

11.) Lines 158-159: I could not find the number of droplets (typically) analyzed for each sample, A, B, Cx. Please provide this information.

12.) Lines 233-234 and Table 1: 'only after 70 mL of washing the ice nucleation activity is entirely lost' There are some data points at temperatures higher than -34°C, both in Figure 4 and Figure S2. Were they ignored in this statement? Also, in Table 1, the CNC values for sample D at -25°C and -34°C are given as zero. Again, I am surprised, because the n_frozen/n_total ratio in Figure 4 and Figure S2 shows values slightly larger than 0. Please elaborate.

13.) Figure 1: It is not clear to me whether the images shown in panels a) and b) and the sketch in panel c) are original to the current work, or whether they have been taken from the given references. I do not understand what is meant in the caption by 'information for the drawing is taken from....'